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14. ABSTRACT

Efforts were made to improve the microbial synthesis of D-1,2,4-butanetriol from D-xylose previously constructed (Scheme 1). Benzoylformate decarboxylase variants from Pseudomonas putida (ATCC 12633) with 2-fold increase in kcat/Km values were generated using directed evolution. Pseudomonas fragi D-xylonate dehydratase was purified and sequenced. E. coli yjhG and yagF encoding D-xylonate dehydratases, and yjhH and yagE genes encoding 3-deoxy-D-glycero-pentulosonate aldolases were identified, respectively. Site-specific knockouts of the aldolases prevented the cleavage of intermediate 3-deoxy-D-glycero-pentulosonic acid to form pyruvic acid and glycolaldehyde. D-Xylose dehydrogenase from Caulobacter crescentus CB15 was identified and expressed in E. coli that converts D-xylose into D-xylonic acid.

15. SUBJECT TERMS

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Final Technical Report
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Performing Organization and Address: Chemistry Department, Michigan State University, E. Shaw Lane, East Lansing, MI 48824
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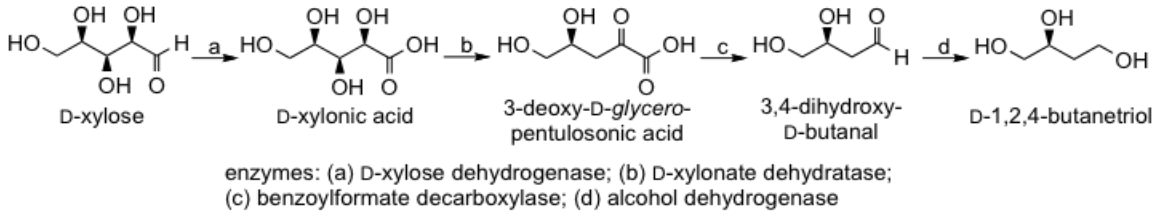
Abstract/Project Summary:

Efforts were made to improve the microbial synthesis of D-1,2,4-butanetriol from D-xylose previously constructed (Scheme 1). Benzoylformate decarboxylase variants from *Pseudomonas putida* (ATCC 12633) with 2-fold increase in k_{cat}/K_m values were generated using directed evolution. *Pseudomonas fragi* D-xylonate dehydratase was purified and sequenced. *E. coli yjhG* and *yagF* encoding D-xylonate dehydratases, and *yjhH* and *yagE* genes encoding 3-deoxy-D-*glycero*-pentulosonate aldolases were identified, respectively. Site-specific knockouts of the aldolases prevented the cleavage of intermediate 3-deoxy-D-*glycero*-pentulosonic acid to form pyruvic acid and glycolaldehyde. D-Xylose dehydrogenase from *Caulobacter crescentus* CB15 was identified and expressed in *E. coli* that converts D-xylose into D-xylonic acid.

Scheme 1.



enzymes: (a) D-xylose dehydrogenase; (b) D-xylonate dehydratase; (c) benzoylformate decarboxylase; (d) alcohol dehydrogenase

Scientific Technical Objectives:

Increasing key enzyme activity and eliminating side product biosynthesis will enhance the concentration and yield of D-1,2,4-butanetriol microbially synthesized from D-xylose. Directed evolution is widely used to generate enzyme varients with improved characteristics. Benzoylformate decarboxylase variants with substrate specificity towards 3-deoxy-D-*glycero*-pentulosonic acid will likely be identified. *E. coli* is known to use D-xylonic acid as a sole carbon source for growth, and the involved native pathway cleaves 3-deoxy-D-*glycero*-pentulosonic acid into pyruvate and glycolaldehyde. Identification and knockout of related genes becomes essential to increase D-1,2,4-butanetriol production. Microbial synthesis D-1,2,4-butanetriol from D-xylose requires D-xylose dehydrogenase activity for the conversion of D-xylose into D-xylonic acid. Identification of such enzyme enable the construction of a single *E. coli* microbe that is capable to convert D-xylose to D-1,2,4-butanetriol.

Approach:

Error-prone PCR and DNA shuffling methods were used to create benzoylformate decarboxylase mutant library. A high throughput colorimetric assay was developed and used to screen for mutant with improved characteristics. D-Xylonate dehydratase from *P. fragi* was purified to homogeneity and its sequence was determined. This sequence in tandem with bioinformatics was to be used to identify D-xylonate dehydratase activity in *E. coli*. Based on the frequent clustering of genes in microbial catabolism, identification of this dehydratase activity led to the

identification of the gene encoding 3-deoxy-D-*glycero*-pentulosonate aldolase. Similarly, The partial DNA sequence of D-xylonate dehydratase was used to search the ERGO database to identify Orfs with 50 – 70% sequence homology. Identified Orfs in *Burkholderia fungorum* LB400 and *Caulobacter crescentus* CB15 were in close proximity to Orfs encoding a short chain dehydrogenase. These sequences were cloned, expressed in *E. coli* and assayed for D-xylose dehydrogenase activity.

Accomplishments:

A high throughput screening method was developed to evaluate the *in vitro* benzoylformate decarboxylase variants towards 3-deoxy-D-*glycero*-pentulosonate. To generate the mutant library, the native *mdlC* gene encoding the wild-type benzoylformate decarboxylase from *P. putida* was first subjected to error-prone PCR. Mutant candidates with improved *in vitro* decarboxylase activities were then recombined using DNA shuffling. Mutant benzoylformate decarboxylases with up to 2-fold increase in k_{cat}/K_m values using 3-deoxy-D-*glycero*-pentulosonate as substrate were identified. Amino acid changes shared by these improved mutants were further identified by DNA sequencing. Genes *yjhG* and *yagF* were discovered to encode two isozymes of D-xylonate dehydratase in *E. coli*. Two isozymes of 3-deoxy-D-*glycero*-pentulosonate aldolase was also identified in *E. coli*, which are encoded by *yjhH* and *yagE*. Site-specific double knockout in the loci encoding the aldolase isozymes was generated. No growth was observed with this *E. coli* mutant when D-xylonic acid was used as the sole source of carbon. The genes encoding proteins RCO01012 from *C. crescentus* CB15 and RBU11704 from *B. fungorum* LB400 was discovered to possess D-xylose dehydrogenase activity. D-Xylose dehyrogenase from *C. crescentus* is the best candidate for expression in *E. coli* to create a construct capable of synthesizing D-1,2,4-butanetriol from D-xylose.

Significance:

A practical route has been established enabling the synthesis of D-1,2,4-butanetriol from D-xylose.

Publications:

None

Patent Information:

Frost, J.W. U.S 2006/0234363, Oct. 19 2006.
Frost, J. W. WO 2008/091288, July 31, 2008.

Technology Transfer:

Technology Licensed to Draths Corporation

Awards/Honors:

None.